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Cover Page Footnote
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PART IV: Pesticides

Chapter 11

USE OF BIOMARKERS AND PREDICTED ENVIRONMENTAL CONCENTRATIONS (PEC) TO SELECT RELEVANT PESTICIDES APPLIED TO SOIL

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ABSTRACT

In our country, many different types of pesticides may be applied to cut flower crops in order to protect them. Two groups of these compounds, organophosphate (OP) and carbamate (CA) are used in high quantities. Both of them produce inhibition of cholinesterase activity in different organisms. This characteristic is used to identify the presence of these compounds with fast tube tests. On the other hand, some lixiviation models like PESTAN and others have been used in ecological risk assessment studies to get the Predicted Environmental Concentrations (PEC) of pesticides in soil. The aim of this research is to determine if PEC of several OP and CA compounds applied to a flower crop area, will show any correlation with inhibition of cholinesterase activity detected in soil extracts. Samples of surface soil (0 – 30 cm in depth) and subsurface soil (30 to 60 cm in depth) were taken from a flower crop area in which, during the last two years, OP pesticides (like acephate, dimetoate and methyl parathion), and CA pesticides (like carbendazim, carbofuran and metomil) were applied. Weekly loads of these pesticides were registered to estimate the annual load of each compound. Physicochemical analysis and relative inhibition of cholinesterasic

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activity were developed for each soil sampled. PEC values were estimated using PESTAN (US-EPA) lixiviation model for each pesticide considering the data of physicochemical analysis of each soil sampled. From all pesticides tested only acephate and metomilo showed a significant correlation ($p < 0.01$) between PEC values and inhibition cholinesterase activity of soil extracts. These results suggest that inhibition of cholinesterase activity observed in soil extracts is produced mainly by these two pesticides. Further studies could be oriented to measure concentrations of acephate and metomil to develop actions to reduce their environmental impact.

Keywords: Pesticides in soil, cholinesterase activity in soils, organophosphorous and carbamate pesticides in soil

1. INTRODUCTION

A problem observed in flower crop areas located in developing countries is the use and application of many kind of pesticide compounds in complex mixtures (Moncada, 2006). Some flower crops get almost ninety loads of pesticides during a six month time space. This represent a meaningful load of pesticides which can be reach the soil and travel through it.

The knowledge of pesticides mobility through soil is very important to identify exposure conditions of non target organisms and to prevent potential contamination of surface water and groundwater resources (Rao and Hornsby, 2001), and health and environmental risks (Ahlers and Martin, 2003; Finizio and Villa, 2002; Villa et al., 2003a).

When complex mixtures of pesticides are present in soils, the quantification of each compound requires a lot of time and it represent a high cost (Villa et al., 2003b), for this reason it is very useful to develop preliminary screening procedures in order to identify which of these compounds are of special interest and select the appropriate methods to identify and quantify them.

Organophosphorate and carbamate compounds are two major group of pesticides used in flower crops in Mexico. Both groups produce inhibitory effects on cholinesterase activity in different organisms, this characteristic has been used to identify the presence of these compounds in the water (Hamers et al., 2000), food (Schulze et al., 2002) or, like in this case, in soils with the use of biosensors and fast tests (Guerrieri et al., 2002; Andreou and Clonis, 2002) . On the other hand, different lixiviation models lixiviation have been applied to determine Predicted Environmental Concentrations (PECs) of these compounds. PECs are values used in ecological risk assessment studies (Villa et al., 2003b; Peterson, 2006).

The aim of this study was to determine if cholinesterase activity inhibition used as a marker of presence of organophosphorate, and carbamate pesticides and detected in soil extracts samples from a greenhouse flower crop area, combined with environmental concentrations estimated (PEC) by lixiviation models like PESTAN (Pesticide Analytical Model Version 4.0, US-EPA) developed by the Center for Subsurface Modeling Support (CSMoS) (Ravi and Johnson, 1986; Stacy et al., 2007), can be used as a useful primary method to identify and select relevant exposure concentrations of these pesticides. PESTAN model is based in an analytical solution of advective-dispersive-reactive transport equation for pollutant movement in soil developed by Enfield et al., 1982, which can be used with an user-friendly interface in the Windows operating system (Stacy et al., 2007).

2. MATERIALS AND METHODS

2.1 Soil Sampling

Georeferenced soil samples of 1 Kg were taken from a greenhouse flower crop area of Gerbera (*Gerbera jamesonii*). Twelve samples from a surface soil fraction (0 to 30 cm in depth) and twelve from a subsurface soil fraction (30 to 60 cm in depth) were sampled using a clay auger (Mason, 1992). Gerbera is an important type of flower crop for exportation with high sales in the international flower market. During 2004 and 2005 years all the loads of pesticides used in this area

Table 1. Annual load of pesticides used in Gerbera crop area which were selected to calculate data of Predictable Environment Concentrations (PECs) by PESTAN model

Pesticide	**Group**	**Load of Pesticide (Kg/ha/año)**	**Year**
Acefate	Organophosphorous	6.68	2004
Carbendazim	Carbamate	0.24	2005
		0.19	2004
Carbofuran	Carbamate	0.51	2004
Dimetoate	Organophosphorous	1.94	2005
		6.96	2004
Metomil	Carbamate	0.43	2005
		0.065	2004
Methyl Parathion	Organophosphorous	0.24	2004

to protect the crops were registered. This information was used to estimate annual load of each pesticide expressed as Kg/Ha/Year (Table 1). Each soil sample was placed in a plastic bag and it was transported to laboratory to develop the physicochemical soil test and the percent of cholinesterase activity inhibition test in a soil extract.

2.2 Soil Physicochemical and Cholinesterase Inhibition Tests

Physicochemical soil test included the measure of pH, texture, composition, conductivity, percent of organic matter and moisture, according to the methods of the Soil Survey Standard Test Methods of Department of Sustainable Natural Resources of Australia, also, according to the normalized Mexican test (NMX): NMX-AA-021-1985, NMX-AA-016-1984, NMX-AA-025-1984, NMX-AA-052-1985 y NMX-AA-015-1985.

To prepare soil extracts, each soil sample was homogenized, and a portion of 20 g of soil was added with 50 mL of acetone, the mixture was shaken during 10 minutes, at the end of this time mixture was filtered through Wattman Num. 1 filter paper, the filtrated solution was collected in a glass beaker, then acetone was evaporated in a water bath at 35°C until dry.

The field kit “In Quest OP/Carbamate Screen” ®, which is used as colorimetric assay for the qualitative detection of organophosphate and carbamate pesticides, was used in order to quantify the cholinesterase activity. In this study, we modified the procedure to enable the results to be read by a spectrophotometer "Thermolyne Spectronics model Genesys-6,"; the measurement of light absorbance affords a semiquantitative measure of enzymatic reaction.

In this case, dry residue was dissolved in a glass beaker with 2.5 mL of OP/Carbamate Screen wash solution A ®, from this solution was taken 1mL to add to buffer/chomogen reagent ® and vigorously shaken in a vortex. This solution was added to a tube with lyophilized acetylcholinesterase, this tube was incubated in a water bath during 20 minutes and then filtered and poured into a quartz cuvette to read the absorbance at 480 nm.

The basic principle underlying this test is the inhibition of the acetylcholinesterase enzyme produced by organophosphate and carbamate compounds. When these pesticides are absent in a soil extract, acetylcholinesterase hydrolyzes a chromogenic ester compound ®, which is used as a substrate and suffers oxidation to become deep blue in color. If these pesticides are present in the extract of soil sample, they produce a decrease of color proportional to pesticide concentration (In Quest OP/Carbamate Screen ® Data sheet of Kit).

The change of color was measured in absorbance units at 480 nm, absorbance of a blank sample without pesticides was considered as 100% of cholinesterase activity or 0% of relative inhibition, absorbance of each soil sample extract was referenced to this parameter as relative enzymatic activity expressed in %. The reduction of this relative activity was expressed as % relative inhibition of cholinesterase activity in a given soil sample. Positive controls were prepared using solutions with known concentrations of carbofuran and metamidophos, two pesticides used commonly in pest control for Gerbera crops.

2.3 Use of PESTAN Model to get PECs of Pesticides

Based on information data about physicochemical characteristics of each soil sample, the annual load (measured in Kg/Ha/Year) of each of the applied pesticides and their physicochemical data and also irrigation conditions as inputs for PESTAN model, data of PECs were calculated for each pesticide.

2.4 Statistical Analysis of Data

Association between physicochemical data and cholinesterase activity measured in different (surface and subsurface) soil samples from the Gerbera crop area were developed by a statistical correlation analysis using rho Spearman coefficient, previously knowing the normality characteristics of the different data. Significant values were taken when correlation was $p < 0.05$. All statistical tests were developed by SPSS v.11 software.

3. RESULTS AND DISCUSSION

3.1 Physicochemical Characterization and Relative Inhibition of Cholinesterase Activity

Tables 2 and 3 show physicochemical data of different soil samples from the Gerbera crop area surface and subsurface fractions, and percent of relative inhibition of cholinesterase activity measured for each soil fraction.

Values of organic carbon and the percent of organic matter were higher in surface soil fractions than in subsurface soil fractions. Also lightly higher values of percent of sand were found in the subsurface soil fraction. Conductivity values were higher in the surface soil fraction than in the subsurface soil fraction.

In some cases, higher percent values of relative inhibition of cholinesterase activity were detected in subsurface soil fractions. The level of relative inhibition of cholinesterase activity measured in surface and subsurface fraction of soil

samples can be considered as a marker of organophosphorous and carbamate pesticides present in soil, which they are compounds that produce inhibition of this enzyme in live organisms.

Non-significant correlation was detected between inhibition levels of cholinesterase activity and physicochemical data from surface and subsurface soil fractions.

Table 4 shows physicochemical properties of some of organophosphourus and carbamate compounds used in the Gerbera crop area. Some of these compounds can be considered as low persistence compounds but their higher frecuency of application and acidic conditions present in soil could produce that some of them have longer life in soils, for example carbendazim, carbofurane and clorpyrifos.

With the pH values form the sampled soils (pH = 5.55 in surface soil samples and pH = 5.51 in subsurface soil samples) it is suggested they could be classified as light acidic soils (NOM-021-RECNAT-2000, 2002). Previous studies have shown that acidic soils can increase persistence of organophosphorous pesticides (Pantelelis et al., 2006), and decrease microbial activity required to breakdown these substances (Nannipieri et al., 2003; Singh and Walker,2006).

Structure of soil describes physical and structural characteristics from it, and these characteristics can determine the movements of water and pesticides solubilized in the soil and some interactions between soil and pesticides (Carter, 2000; Ciglasch et al., 2005).

According to the percent of sand, silt or clay presented in the diferent samples of soil, surface and subsurface soil fraction were clasified as clay-loam and loam soil texture respectively. A higher percent of sand could represent more filtering capability and some ionic and soluble pesticides like metamidophos and metomil could move to the subsurface soil fraction and explain the values of relative inhibition of cholinesterase activity measured in this level.

A significant correlation between % of clay in surface fraction with % of clay in subsurface fraction was observed ($R = 0.779$; $p < 0.05$), also significant correlation between % of moisture in the two fractions and % of sand ($R = 0.703$; $p < 0.05$), suggesting homogeneity of two layers of soil. The highest correlation was observed between values of relative inhibition in percent of cholinesterase activity measured in surface soil fraction with values of percent of relative inhibition of cholinesterase activity measured in subsurface soil fraction (rho = 0.881; $p < 0.001$).

3.2 Modeling Lixiviation of Pesticides used in Gerbera Crop Area with PESTAN to get PECs Values

To get PECs values of each pesticide we consider a scenario with a recharge value of 0.0134 cm/h, based in pluvial precipitation recived during a year, according data registered from two meteorological stations nearest to greenhouse area. Tables 5 and 6 show results of PECs values calculated with PESTAN model considering a recharge of 0.0134 cm/h.

Correlation coefficients of Spearman (rho) between PECs and relative inhibition in percent of cholinesterase activity were determined using SPSS statistical program and are shown in Table 7. It was observed that only PECs of acefate estimated at 100 days show a significant correlation (rho = 0.734; $p < 0.01$) against values of relative inhibition in percent of cholinesterase activity measured in the subsurface soil fraction (30 to 60 cm in depth).

Also, PECs of metomil obtained at 60 days from the surface soil fraction showed significant correlation (rho = 0.734; $p < 0.05$), against of relative inhibition in percent of cholinesterase activity measured in the subsurface soil fraction (30 to 60 cm in depth). These values sugest that pesticides acefate and metomil contribute in first place, to produce the inhibitory effect of cholinesterase activity observed in subsurface soil fraction.

Transport of pesticides in soil involved complex fenomena and their movement is related with water migration through soil which is important to soluble pesticides (Wang et al., 2004), but chemical sorption and degradability could be important also to pesticides with low solubility (Zhang et al., 2000). In the first case the water flux can influence the translocation of pesticides like acefate and metomil located in surface soil fraction move to subsurface soil fraction (Ciglasch et al., 2005), this flux can be facilitaded when there is a major content of sand in soil fraction (Rao and Hornsby, 2001).

Bioassays developed with different organisms like cladocerans (Barata et al., 2007), earthworms (Caselli et al., 2006; Denoyelle et al., 2007) and others have been used in ecotoxicological studies to identify water and soil pollution produced by pesticides. The use of cholinesterase activity inhibition as biomarker of effects of organophosphorous an carbamate pesticides in these bioassays is well recognized. In some cases this characteristic is used to classify the risk of polluted areas (Hagger et al., 2008).

Table 2. Physicochemical test results from surface samples of soil (0 - 30 cm in depth) from crop area of *Gerbera jamesonii*.

Num sample	**Moisture %**	**pH Water**	**pH Ca^{++}**	**Organic carbon %**	**Organic matter %**	**Sand %**	**Clay %**	**Silt %**	**Texture**	**Conductivity** (µS/cm)	**% Inhibition Cholinesterase**
1	4.55	5.97	5.37	1.12	1.94	40.2	24	35.8	Loam	1082	24.79
2	4.00	5.88	5.48	0.83	1.43	30.4	37.8	31.8	Clay-loam	317	4.48
3	4.42	6.02	5.77	0.95	1.64	32.4	38	29.6	Clay-loam	1167	32.50
4	4.01	5.90	5.44	1.55	2.68	30.4	35.8	33.8	Clay-loam	651	42.19
5	3.57	5.39	4.91	1.32	2.27	38.4	29.8	31.8	Clay-loam	406	37.50
6	3.81	5.14	4.69	1.45	2.51	34.4	30	35.6	Clay-loam	552	20.94
7	3.86	5.90	5.44	1.88	3.25	40.2	24	35.8	Loam	1860	10.21
8	3.50	6.03	5.49	1.14	1.97	48.4	20	31.6	Loam	368	29.90
9	3.77	6.41	5.98	1.18	2.04	38.2	36	25.8	Clay-loam	1119	39.17
10	3.11	7.15	6.45	0.99	1.70	40.4	24	35.6	Loam	390	25.63
11	4.11	6.42	5.89	0.99	1.70	36.4	32	31.6	Clay-loam	479	67.71
12	3.57	6.55	5.72	1.24	2.14	40.4	32	27.6	Clay-loam	566	54.48
Mean	**3.86**	**6.06**	**5.55**	**1.22**	**2.11**	**37.5**	**30.2**	**32.2**	**Clay-loam**	**746**	**32.45**
S.D.	0.39	0.52	0.46	0.29	0.51	5.12	6.10	3.32		466.1	17.62

Table 3. Physicochemical test results from subsurface samples of soil (30-60 cm in depth) from crop area of *Gerbera jamesonii*.

Num sample	**Moisture %**	**pH Water**	**pH Ca^{++}**	**Organic carbon %**	**Organic matter %**	**Sand %**	**Clay %**	**Silt %**	**Texture**	**Conductivity** (μS/cm)	**% Inhibition Cholinesterase**
1	8.0142	6.19	5.68	0.4056	0.6993	60.4	8	31.6	Sandy-loam	564	30.00
2	3.7401	5.88	5.38	1.2441	2.1448	32.4	33.8	33.8	Clay-loam	402	0.00
3	4.9855	6.22	5.76	0.3276	0.5648	34.4	32	33.6	Clay-loam	1160	41.98
4	4.5040	5.78	5.29	0.9321	1.6069	34	28	38	Clay-loam	335	50.10
5	4.0218	5.7	5.07	0.7956	1.3716	36.2	26	37.8	Loam	211	44.06
6	4.9459	5.75	5.25	0.8736	1.5061	42.2	22	35.8	Loam	301	38.33
7	4.6738	5.3	5.02	0.9516	1.6406	38.2	26	35.8	Loam	778	24.79
8	4.0473	6.11	5.61	0.8151	1.4052	54.4	16	29.6	Sandy-loam	417	35.63
9	6.1571	6.52	5.75	0.5031	0.8673	42.4	30	27.6	Clay-loam	425	37.40
10	2.2900	6.34	5.58	0.1911	0.3295	40.4	20	39.6	Loam	230	28.54
11	4.9188	6.6	6.02	0.7566	1.3044	42.4	35.6	22	Clay-loam	681	63.33
12	4.4557	6.52	5.75	0.4056	0.6993	42.4	29.8	27.8	Clay-loam	291	48.65
Mean	**4.72**	**6.07**	**5.51**	**0.68**	**1.17**	**41.6**	**25.6**	**32.7**	**Loam**	**482.9**	**36.90**
S.D.	1.38	0.39	0.31	0.31	0.54	8.2	7.9	5.2		275.5	15.71

Table 4. Physicochemical properties* of organophosphate and carbamate pesticides inhibitors of cholinesterase activity used to control pests in crop area of *Gerbera jamesonii*.

Active Chemical	No. CAS	Chemical Group**	Use***	Clas.Tox. (WHO)	Molecular Weight	Water Solubility	Vapor Pressure	Partition Coefficient (Kow)	Adsorption Coefficient (Koc)	T ½ Soil
Acefate	30560-19-1	OP	I	III	183.17	79 g/100mL to 20 °C; 650 g/L to 20°C	2.3 x 10 E-6 mbar a 24°C; 1.7 x 10 E-6 mmHg to 24°C	-1.87	0.48	< 3 to 6 days in anaerobic and aerobic soils respectively
Carbendazim	10605-21-7	CA	F	III	191.2	0.0008 g/100 mL to 24°C; 8 mg/L to pH 7	< 100nPa A 20°C	1.49	1900	320 days (Colombia Ministery)
Carbofurane	1563-66-2	CA	I, N	Ia - Ib	221.25	320 mg/L@ 25°C	2.7 mPa @ 33°C	1.23 - 1.41	22	30 a 120 days
Chlorpiriphos	2921-88-2	OP	I	Ib	350.62	2 mg/L @ 25°C	2.5 mPa @ 25°C	4.699	6070	11 a 141 days
Diclorvos	62-73-7	OP	I	Ia	220.98	0.8 g/ 100 mL to 20 °C	290 mPa @ 20°C (1.6 Pa a 20 °C, ICSC)	1.47	30	7 days
Dimethoate	60-51-5	OP	I	Ib	229.28	25 g/L @ 21°C	1.1 mPa @ 25 °C	0.699	20	20 days
Metomil	16752-77-5	CA	I	Ia	162.21	57.9 g/L @25°C	6.65 mPa @ 25°C	0.6	72	14 days
Monocrotophos	2157-98-4	OP	I	Ia	223.2	Soluble in water (100 g/100 mL of water to 20°C)	2.9 x 10 E-1 mPa 20°C (0.0003 Pa a 20°C)	-0.22	ND	7 days in soil exposed to sun light
Oxamyl	23135-22-0	CA	I	Ib	219.25	Soluble in water	14.1 mm Hg a 20°C (31 mPa a 25°C Extoxnet)		0.15	10.7 days (5.73 days in anaerobic soil) 4 a 20 days (Extoxnet)
Methyl Parathion	298-00-0	OP	I	Ia	263.21	55-60 mg/L @25°C	1.3 mPa 20 °C	3.51 - 3.83	5100	1 a 30 days with a representative value of 5 days

(*) All data were obtained from specialized databases: PAN, EXTOXNET, ICSC, NIOSH, IRIS, and Security data sheet (MSDS)
(**) OP Organophosphate, CA Carbamate; (***) I, Insecticide; F, Fungicide; N Nematicid

In the same way, during the last twenty years, many devices of biosensors (Andreescu and Marty, 2006) and bioanalytical test (Luque de Castro and Herrera, 2003) based on cholinesterase inhibition enzyme have been used for environmental monitoring (Rodriguez-Mozaz et al., 2005) and developed to identify and quantify exposure to organophosphorous and carbamate compounds in food (Amine et al., 2006), water (Arduini et al., 2006) and soil (Velazco-García and Mottram, 2003).

In the present study, relative cholinesterase activity inhibition observed in soil extract samples can be considered as a preliminary test to identify inhibitory compounds of this enzyme. The relative inhibition of cholinesterase activity observed in the surface and subsurface soil fractions is a marker of inhibitory substances in this case organophosphorous and carbamate compounds present in the complex mixture of pesticides applied in the flower crop area.

When combine these results of cholinesterase inhibition with PEC values determined by PESTAN ® model for the different pesticides and only the PEC values of acefate and metomil presented a good correlation, this suggest a promissory application to identify witch of the different pesticides applied in crop areas are responsible of this effect and also to identify possible hot spots of these compounds present in a crop area.

In PESTAN model, vertical transport of solved pollutants through vadose zone of soil is simulated as polluted water block which migrates into an homogeneous soil. The polluted block starts its travel through soil when the first precipitation happens at a rate equal to water pore rate. Once the polluted block is into soil, transport of pollutant is influenced by sorption and dispersion process (Ravi and Johnson, 1986; Stacy et al., 2007).

The relative inhibition observed is not specific to any of the applied pesticides, however, by setting the correlation with the PEC acquired by the PESTAN simulation model, it was possible to see that only some of the applied pesticides (acetafe and metomil) showed a meaningful correlation ($p < 0.01$) against the values of relative inhibition of cholinesterase activity evaluated values.

This selectivity can be explained considering that organophosphorous and carbamate pesticides will be moving through soil at different rate depending on their own physicochemical characteristics, for example solubility, affinity to organic matter of soil (Koc) and others (Sun et al., 2008; Franco and Trapp, 2008) and physicochemical properties of soil, for example texture, porosity, organic matter content, clay, and others (Wang el al., 2004).

In this case acefate and metomil are water soluble pesticides. When we take account the preparation of soil extract with acetone, we consider that almost all

Table 5. Predictable Environment Concentrations (PECs) of pesticides used in Gerbera crop area, data estimated by PESTAN model
Inputs for scenario: surface soil fractions (0 to 30 cm in depth); recharge = 0.0134 cm/h

Pesticide (distance to soil surface = 30 cm)	**Time considered for lixiviation (days)**	**PECs (ppb) calculated for each sample point**											
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Metomil	60	17.54	32.69	18.06	1.6	1.66	1.66	0	3.33	4.95	13.26	14.79	3.31
	100	0	119.29	103.17	27.67	43.69	34.12	6.63	76.53	69.05	113.87	94.67	56.39
	500												
Carbofuran	60	22.92	14.63	12.95	15.93	15.89	15.99	25.27	28.26	15.18	23.79	13.65	15.60
	100	0.947	0.943	0.544	2.59	1.80	2.33	8.50	1.38	1.17	0.630	0.675	1.44
Dimetoate	60	0.660	35.15	40.51	55.28	53.36	54.95	101.21	89.82	49.41	72.13	43.09	51.50
	100	0.00018	0.806	1.272	6.496	4.390	5.794	21.54	2.964	2.814	1.306	1.588	3.491
Acefate	100	13.73	65.96	79.47	121.47	93.96	121.27	13.88	13.99	93.46	13.66	79.63	107.08
	500	0	0	0	0	0	0	0	0	0	0	0	0
Carbendazim	100	0	0	0	0	0	0	0	0	0	0	0	0
	500	0	0	0	0	0	0	0	0	0	0	0	0

Table 6. Predictable Environment Concentrations (PECs) of pesticides used in Gerbera crop area, data estimated by PESTAN model
Inputs for scenario: subsurface soil fractions (30 to 60 cm in depth), distance to soil surface = 60 cm; recharge = 0.0134 cm/h

Pesticide (Distance to soil surface in cm)	**Time considered for lixiviation (days)**	**PECs (ppb) calculated for each sample point**											
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Metomil (60)	100	51.40	1.55	74.12	1.55	1.58	3.16	1.60	1.61	15.26	193.47	1.56	39.13
Metomil (30)	100	84.11	59.56	77.08	102.32	147.96	135.64	123.25	152.50	120.62	10.22	125.67	102.35
Carbofuran (30)	60	5.17	15.53	4.10	13.08	16.81	20.18	32.31	22.65	6.79	1.30	10.63	5.28
	100	0.0054	1.394	0.0224	0.565	0.202	0.357	0.505	0.222	0.0769	0.00054	0.273	0.041
Carbofuran (60)	60	5.56	0.026	0.989	0.110	0.772	0.462	0.319	0.931	0.600	4.347	0.244	0.803
	100	0.596	0.772	0.245	0.827	1.998	2.324	2.477	3.424	0.460	0.0703	0.728	0.340
Dimetoate (60)	60	20.83	0.159	3.51	0.565	3.745	2.446	1.788	5.041	2.336	14.28	1.090	2.960
	100	1.356	2.698	0.666	2.573	5.520	6.737	7.431	9.378	1.269	0.176	2.127	0.925
Dimetoate (30)	60	13.64	51.13	12.32	40.97	48.73	59.67	66.94	64.14	20.37	3.74	32.47	15.83
	100	0.0105	3.358	0.0547	1.325	0.412	0.732	1.046	0.413	0.181	0.00123	0.634	0.098
Acefate (60)	100	78.83	1752.7	1361.4	1643.8	438.7	680.5	508.6	134.0	1448.9	219.8	1553.8	1404.6
	500	0	0	0	0	0	0	0	0	0	0	0	0
Acefate (30)	100	0	93.6	51.8	79.5	13.3	12.6	13.6	14.6	52.2	14.0	79.0	51.0
Acefate (90)	100	1984.1	1003.4	1555.8	1192.5	2741.1	2743.0	2739.0	2513.1	1422.8	2470.0	1290.5	1495.7
Carbendazim	100	0	0	0	0	0	0	0	0	0	0	0	0
	500	0	0	0	0	0	0	0	0	0	0	0	0

Table 7. Non parametric correlation values (rho) between PECs calculated by PESTAN model and percent of relative inhibition of cholinesterase activity measured in surface and subsurface soil fractions from Gerbera crop area. Recharge value = 0.0134 cm/h

Pesticide (Scenario)	Correlation value (rho) with surface cholinesterase inhibition	p <	Correlation value (rho) with subsurface cholinesterase inhibition	p <
Carbofuran (100 days, surface)	-0.070	NS	0.098	NS
Carbofuran (60 days, surface)	-0.420	NS	-0.441	NS
Carbofuran (30 cm, 100 days, subsurface)	-0.203	NS	-0.007	NS
Carbofuran (subsurface cm, 60 days, subsurface)	-0.315	NS	-0.133	NS
Carbofuran (60 cm, 100 days, subsurface)	-0.308	NS	-0.098	NS
Carbofuran (60 cm, 60 days, 1subsurface)	-0.014	NS	-0.140	NS
Acefate (30 cm, 100 days, surface)	**0.517**	**NS**	**0.734**	**0.01**
Acefate (60 cm, 100 days, subsurface)	0.301	NS	0.294	NS
Acefate (30 cm, 100 days, subsurface)	**0.517**	**NS**	**0.734**	**0.01**
Dimetoate (100 days, surface)	-0.140	NS	0.294	NS
Dimetoate (60 days, surface)	-0.077	NS	-0.084	NS
Dimetoate (60 cm, 100 days, subsurface)	-0.371	NS	-0.168	NS
Dimetoate (60 cm, 60 days, subsurface)	-0.077	NS	-0.154	NS
Dimetoate (30 cm, 100 days, subsurface)	-0.203	NS	-0.007	NS
Dimetoate (30 cm, 60 days, subsurface)	-0.364	NS	-0.189	NS
Metomil (100 days, surface)	0.301	NS	0.294	NS
Metomil (60 days, surface)	**0.517**	**NS**	**0.734**	**0.01**
Metomilo (30 cm, 100 days, subsurface)	0.056	NS	0.224	NS
Surface Cholinesterase	1.0	-	**0.881**	**0.01**
Subsurface Cholinesterase	**0.881**	**0.01**	1.0	-

organophosphorous and carbamate pesticides are more soluble in acetone than in water. This gently extraction was useful to through out some of the more soluble pesticides present in the complex mixture.

4. CONCLUSION

Measures of inhibition cholinesterase activity levels can be considered like an instant photographic picture taken in a specific time of an inhibitory substance or substances present in a soil fraction taken in a specific time. The same occurs when a chemical analysis of a pesticide is developed in a soil sample, in this case a selected pesticide is monitoring through concentrations in field.

Our results suggests that measure of the relative inhibition of cholinesterase activity measured in a soil extract, associated with the predicted environmental concentration (PEC) acquired by the PESTAN simulation could be used to identify pesticides organophosphorous or carbamate compounds presented in a complex mixture which are responsible of inhibition observed and prioritize which of them need to be analyzed by chemical methods in further studies.

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